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Arm-Gal4 inheritance influences development and lifespan in *Drosophila melanogaster*

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ABSTRACT. The *UAS-Gal4* ectopic expression system is a widely used and highly valued tool that allows specific gene expression in *Drosophila melanogaster*. Yeast transcription factor Gal4 can be directed using *D. melanogaster* transcriptional control elements, and is often assumed to have little effect on the organism. By evaluation of the consequences of maternal and paternal inheritance of a *Gal4* transgene under the transcriptional regulation of *armadillo* control elements (*arm-Gal4*), we demonstrated that Gal4 expression could be detrimental to development and longevity. Male progeny expressing *arm-Gal4* in the presence of *UAS-lacZ* transgene had reduced numbers and size of ommatidia, compared to flies expressing *UAS-lacZ* transgene under the control of other Gal4 transgenes. Aged at 25°C, the median life span of male flies with maternally inherited *elav-Gal4* was 70 days, without a responding transgene or with *UAS-lacZ*. The median life span of maternally inherited *arm-Gal4* male flies without a responding transgene was 48 days, and 40 days with the *UAS-lacZ* transgene. A partial rescue of this phenotype was observed with the expression of *UAS-lacZ* under paternal *arm-Gal4* control, having an average median lifespan of 60 days. This data suggests that *arm-Gal4* has detrimental effects on *Drosophila* development and lifespan that are directly dependent upon parental inheritance, and that the benign responder and

reporter gene *UAS-lacZ* may influence *D. melanogaster* development. These findings should be taken into consideration during the design and execution of *UAS-Gal4* expression experiments.

Key words: *Drosophila melanogaster*; *Arm-Gal4*; Parental inheritance; *UAS-lacZ*; Development; Longevity

INTRODUCTION

In *Drosophila melanogaster,* the *UAS-Gal4* bipartite ectopic expression system is an extremely useful tool in genetic manipulations, allowing directed gene expression in a temporal and tissue-specific manner. Based on the transcriptional activator *Gal4* from *Saccharomyces cerevisiae*, the system depends upon Gal4 protein binding to upstream activating sequence (UAS) transcriptional control elements to drive gene expression (Brand and Perrimon, 1993). In yeast, the *UAS* sequence is located near the genes it regulates, *Gal10* and *Gal1*, and transcription of the genes is induced upon binding between Gal4 protein and the UAS (Duffy, 2002). This system allows for the precise manipulation of genes and genetic regulation, making a number of genetic experiments possible.

The *Gal4* transgenes, under the control of a wide range of *Drosophila* control elements, as well as an ever-expanding number of *Drosophila* and other species' transcription units adjacent to the UAS control elements, are maintained in distinct lines. The mating of specific *Gal4* and *UAS* lines results in gene expression in progeny through Gal4 transcription factor binding to UAS, to direct expression (Phelps and Brand, 1998). This method of conditional expression is particularly important when investigating genes that have harmful consequences upon expression.

As the yeast UAS elements are not found in the flies, it has been widely assumed that *Gal4* transgenes have little or no negative effects upon *Drosophila melanogaster* (Brand and Perrimon, 1993). However, it is becoming apparent that caution must be exercised, as some instances in which Gal4 has had an effect have been noted. Under certain experimental conditions, *Gal4* affects ommatidial development and increases apoptosis in the *D. melanogaster* eye when expressed under the glass multiple reporter (*GMR*) promoter element (Freeman, 1996; Kramer and Staveley, 2003). Clearly, the Gal4 system has the potential to cause detrimental effects under certain conditions, and this must be considered when conducting experiments using this system.

The *D. melanogaster* eye develops in a precise pattern, resulting in the production of a hexagonal pattern of 750 to 800 ommatidia (Frankfort and Mardon, 2002). Each ommatidium contains eight photoreceptors, or photosensory neurons, and is associated with accessory cells and a four-cell mechanosensory bristle organ. The consistency of formation makes the eye a very useful tissue in which to study subtle aspects of development.

The *armadillo* (*arm*) gene, the *D. melanogaster* homolog of *beta-catenin*, acts as a segment polarity gene during development (Sanson et al., 1996). *Arm* is multifunctional and is involved in intracellular signaling, cytoskeletal regulation, and cell adhesion during embryogenesis (Peifer et al., 1993; Cox et al., 1996; Sanson et al., 1996). Appropriate regulation of *arm* expression during embryogenesis and development of *D. melanogaster* is crucial. The *arm-Gal4* transgene expresses *Gal4* in response to the transcriptional control of *arm* and is ubiquitous in embryos and imaginal discs of *D. melanogaster* (Sanson et al., 1996). A number of comprehensive studies of *D. melanogaster* development and neurogenesis have been conducted using the *arm-Gal4*

transgene, in eye development analyses (Rahman et al., 2013); longevity assays (Rogina and Helfand, 2004; Radyuk et al., 2009; Zid et al., 2009); mRNA expression analyses (Wang et al., 2005); and to investigate other aspects such as oxidative stress (Tsuda et al., 2007) and larval expression patterns (Sen et al., 2010). For the most part, these experiments seemed to rely upon *arm-Gal4* having little to no effect upon the organism. With this in mind, the aim of the current study was to examine the effects of *arm-Gal4* on eye development and longevity. Results presented here indicate that *arm-Gal4* may affect the development, lifespan and cellular processes of *D. melanogaster* and suggest a need to reevaluate previously established data.

MATERIAL AND METHODS

Drosophila melanogaster stocks, media, and culture

Stocks of *UAS-lacZ* responder line (Brand et al., 1994) and *arm-Gal4* (Sanson et al., 1996) and *elav-Gal4* transgenes (Lin and Goodman, 1994) were obtained from the Bloomington Stock Centre (University of Indiana, Bloomington). Stock of w^{1118} was generously provided by Dr. Howard Lipshitz, of the University of Toronto.

Flies were maintained on standard media containing 65 g/L cornmeal; 15 g/L yeast; 5.5 g/L agar; and 0.05% fancy grade molasses, in water supplemented with 5 mL 0.1 g/mL methyl paraben in ethanol, and 2.5 mL propionic acid. Flies were cultured at 25°C. Males possessing the *arm-Gal4* transgene were crossed to *UAS-lacZ* and w^{1118} females. Females possessing the *arm-Gal4* transgene were crossed to *UAS-lacZ* and w^{1118} males.

Mating crosses were set up by placing three to five virgin females and two to five males of the appropriate genotypes together on standard media. To increase the number of progeny, parental flies were re-brooded on new media at days 2, 4 and 6 after being mated. Critical class males were selected.

Scanning electron microscopy

Critical class male progeny were isolated on the day of eclosion and allowed to mature on standard media for three to five days at 25°C. Flies were then placed in 1.5 mL microcentrifuge tubes and frozen at -80°C. To prepare for scanning electron microscopy (SEM), flies were mounted on aluminum studs, with the left eye facing upwards. Mounted flies were desiccated for 24 to 48 hours, and their eyes photographed at 541X magnification, using a (MLA) 650FEG microscope (FEI in Hillsboro, Oregon, USA).

Micrographs were analyzed using ImageJ analysis software (Abramoff et al*.,* 2004). The number of ommatidia and bristles were determined through counting; the ommatidia area was determined using three samples from each eye. A sample consisted of measuring the area of seven ommatidia and dividing by seven. Results were analyzed using GraphPad Prism version 5c (GraphPad Software, Inc. in San Diego, California, USA). An unpaired two-tailed *t*-test was used to determine significance at a level of $P < 0.05$.

Longevity assay

Critical class male progeny were isolated on the day of eclosion and maintained on standard media at 25°C. To avoid overcrowding, flies were kept in cohorts of 25 or less and were

transferred to fresh media every two to four days to ensure ideal conditions. Vials were scored every two days for the presence of deceased flies. Data were entered into GraphPad Prism to generate survival curves, which were analyzed using a Mantel-Cox test with a significance level of $P < 0.05$.

RESULTS

Inheritance patterns of *arm-Gal4* influence eye development

Compared to flies expressing the benign responder under the control of paternally-inherited *da-Gal4, P[GawB]*$^{L(3)31\text{-}1}$ or *GMR-Gal4* (Mawhinney and Staveley, 2011), the number and size of ommatidia were reduced in critical class male progeny expressing *arm-Gal4*, inherited either maternally or paternally, in the presence of the "benign" *UAS-lacZ* responding transgene (Table 1). A slight reduction in the size and number of ommatidia was observed for flies inheriting *arm-Gal4* paternally with no responder, compared to the reciprocal cross progeny; bristle number was not significantly different (Figure 1). No change in eye development was observed when maternally-inherited *arm-Gal4* was in the absence of a responding gene or the presence of a benign responder. When *arm-Gal4* was paternally inherited, males expressing *UAS-lacZ*, compared to no responder, had an increased ommatidia and bristle number, but no significant change in ommatidia size (Figure 1; Table 1). No significant change was observed between flies expressing the benign responder with maternal or paternal *arm-Gal4*.

Table 1. Summary of the effect of *arm-Gal4* inheritance on the *Drosophila* compound eye, with or without the *UAS-lacZ* transgene.

Genotype	Ommatidia area (μm^2)	Ommatidia No.	Bristle No.
arm-Gal4/+ maternal *arm-Gal4* no responder (N = 11)	203.7 ± 5.1	662.1 ± 14.8	496.2 ± 12.7
arm-Gal4/UAS-lacZ maternal *arm-Gal4* benign responder (N = 10)	203.4 ± 4.6	690.5 ± 12.7	528.6 ± 11.2
arm-Gal4/+ paternal *arm-Gal4* no responder (N = 13)	187.1 ± 5.0	590.2 ± 12.4	464.7 ± 11.7
arm-Gal4/UAS-lacZ paternal *arm-Gal4* benign responder (N = 10)	195.9 ± 2.9	700.7 ± 10.1	549.4 ± 8.5
da-Gal4/UAS-lacZ paternal *da-Gal4* benign responder (N = 10)*	216 ± 3	726 ± 8	N/D
GawB$^{l(3)31\text{-}1}$*/UAS-lacZ* paternal *GawB*$^{l(3)31\text{-}1}$ benign responder (N = 10)*	217 ± 3	736 ± 5	N/D
GMR-Gal4/UAS-lacZ paternal *GMR -Gal4* benign responder (N = 10)*	211 ± 2	731 ± 10	N/D

Values are reported as means ± SEM. N/D is not determined. *Data from a previous study (Mawhinney and Staveley, 2011).

arm-Gal4 expression reduces lifespan

The presence of the X-linked, and necessarily maternally inherited, *elav-Gal4* transgene, both when driving the expression of a benign responding gene (*UAS-lacZ*) and with no responder, presented a median lifespan of approximately 70 days. When the same benign transgene (*UAS-lacZ*) and no responding gene was present, in combination with a maternally-inherited *arm-Gal4*, reduced median life spans of, respectively, 42 and 48 days were observed (Figure 2A; Table 2). When the *arm-Gal4* transgene was paternally inherited in the absence of a responding transgene, a reduced median lifespan of 38 days was observed. However, when the *arm-Gal4* transgene was paternally inherited in the presence of the benign *UAS-lacZ* transgene, a median lifespan of nearly 60 days was observed, a significant overall suppression of the reduced lifespan when compared to the other three categories (Figure 2B; Table 3).

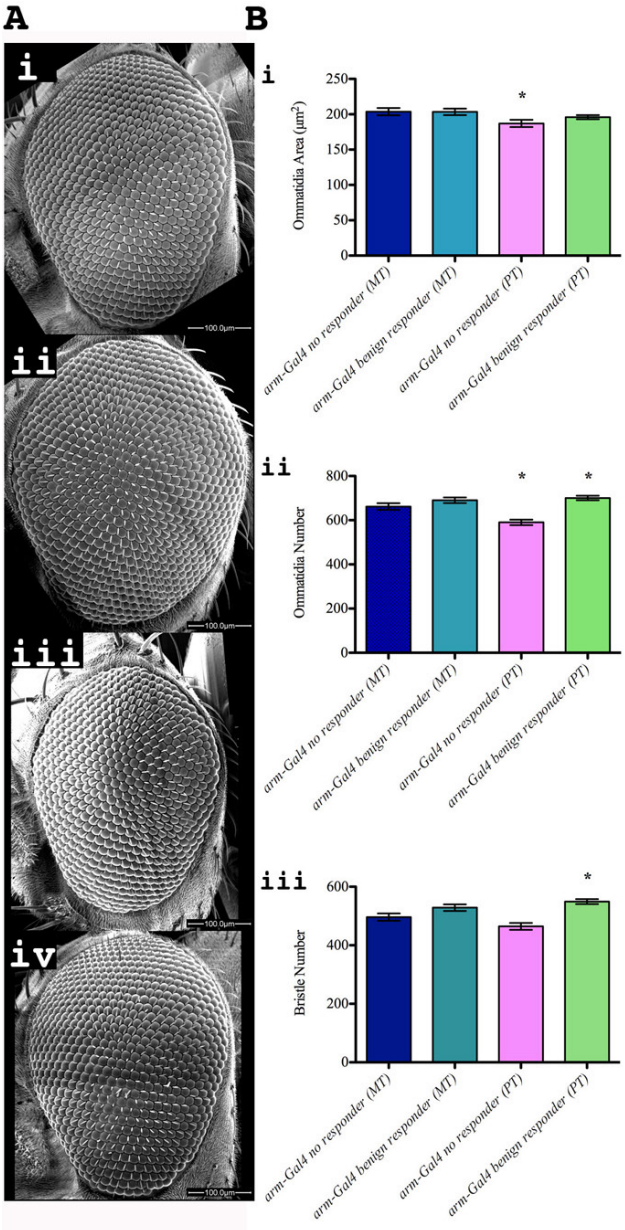

Figure 1. Biometric analysis of eye development influenced by maternal and paternal inheritance of *arm-Gal4* in the presence or absence of *UAS-lacZ*. MT represents maternal and PT represents a paternal inheritance of the *arm-Gal4* transgene. **A.** Scanning electron micrographs of the left eye of adult male *Drosophila melanogaster*. Genotypes are i = w^{1118}; *arm-Gal4/+* (maternal *Gal4*); ii = w^{1118}; *arm-Gal4/UAS-lacZ*; (maternal *Gal4*); iii = w^{1118}; *arm-Gal4/+* (paternal *Gal4*); iv = w^{1118}; *arm-Gal4/UAS-lacZ*; (paternal *Gal4*). **B.** Bar graphs of i, ommatidia area; ii, ommatidia number; and iii, bristle number. Genotypes are: *arm-Gal4* no responder (w^{1118}; *arm-Gal4/+*; MT: N = 11; PT: N = 13) and *arm-Gal4* benign responder (w^{1118}; *arm-Gal4/UAS-lacZ*; MT: N= 10; PT: N = 10). Error bars represent standard error of the mean. *Indicates a significant difference.

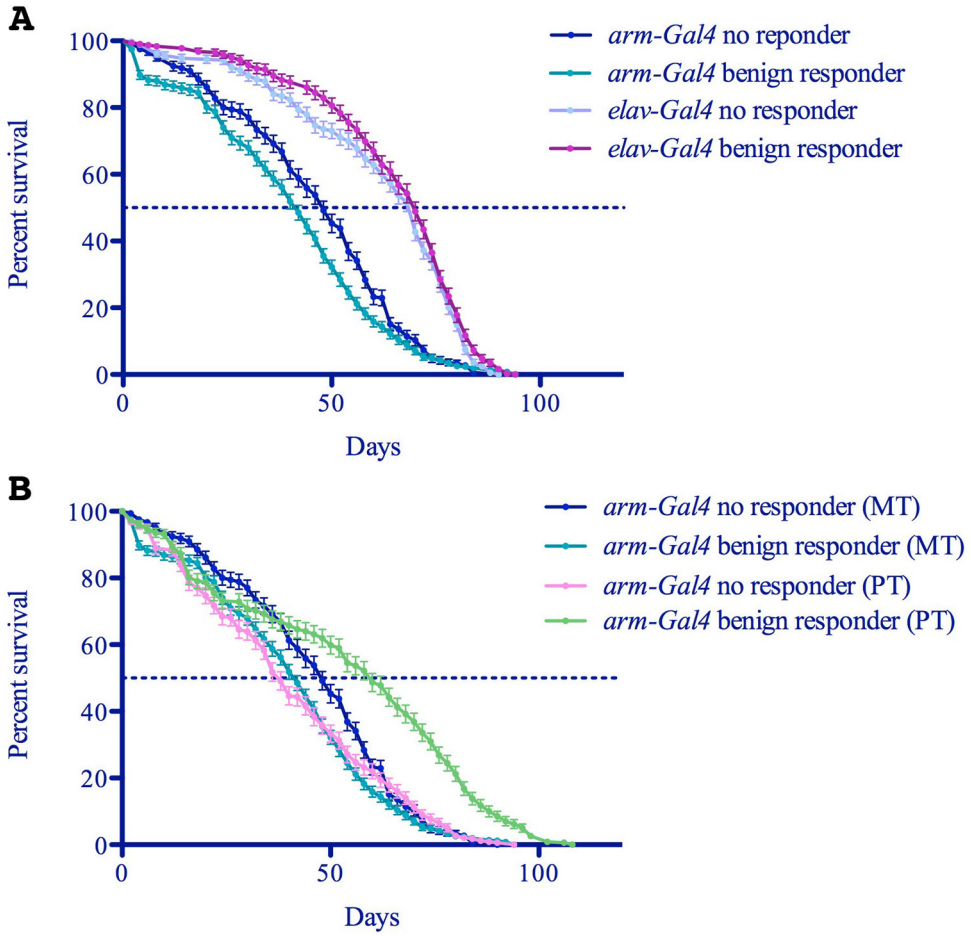

Figure 2. Longevity assay of *Drosophila melanogaster* males influenced by the maternal and paternal inheritance of *arm-Gal4* in the presence or absence of *UAS-lacZ.* Genotypes are: (**A**), maternal *arm-Gal4* with no responder (w^{1118}, *arm-Gal4/+*, N = 331), or a benign responder (w^{1118}, *arm-Gal4/UAS-lacZ*, N = 509) and maternal *elav-Gal4* with no responder (w^{1118} *elav-Gal4, +/+*, N = 365) or a benign responder (w^{1118} *elav-Gal4, UAS-lacZ/+*, N = 315); and (**B**), *arm-Gal4* with no responder (w^{1118}, *arm-Gal4/+*, maternal N = 331, paternal N = 336) or a benign responder (w^{1118}, *arm-Gal4/ UAS-lacZ*, */+*, maternal N = 509, paternal N = 336). Longevity is shown as percent survival ($P < 0.05$, determined by log rank). The dotted line represents median survival of flies. Error bars represent standard error of the mean. MT indicates a maternally-inherited and PT, a paternally-inherited *arm-Gal4* transgene.

Table 2. Longevity of *Drosophila* males with maternally-inherited *elav-Gal4* or *arm-Gal4*, with or without the *UAS-lacZ* transgene.

Genotype	Flies analyzed (N)	Median (50%) survival (days)	Maximum lifespan (days)	P value (compared to *elav-Gal4* no responder)
elav-Gal4; + maternal *elav-Gal4* no responder	365	70	90	-
elav-Gal4; UAS-lacZ maternal *elav-Gal4* benign responder	315	70	94	0.0301
arm-Gal4/+ maternal *arm-Gal4* no responder	331	48	90	<0.0001
arm-Gal4/UAS-lacZ maternal *arm-Gal4* benign responder	509	42	94	<0.0001

Survival curves analyzed using the log-rank test.

Table 3. Longevity of *Drosophila* males, with *arm-Gal4* inherited either maternally or paternally, with or without the *UAS-lacZ* transgene.

Genotype	Flies analyzed (N)	Median (50%) Survival (days)	Maximum lifespan (days)	P value (compared to maternal *arm-Gal4* no responder)
arm-Gal4/+ maternal *arm-Gal4* no responder	331	48	90	-
arm-Gal4/UAS-lacZ maternal *arm-Gal4* benign responder	509	42	94	0.0028
arm-Gal4/+ paternal *arm-Gal4* no responder	336	38	94	0.0688
arm-Gal4/UAS-lacZ paternal *arm-Gal4* benign responder	339	60	108	<0.0001

Survival curves analyzed using the log-rank test.

DISCUSSION

Analysis of the eyes of *D. melanogaster* possessing a paternally-inherited *arm-Gal4* transgene, along with the "benign responder" *UAS-lacZ*, revealed a reduction in ommatidial growth, compared to data from previous studies with *da-Gal4, GMR-Gal4* and *P[GawB]*$^{L(3)31\text{-}1}$ (Mawhinney and Staveley, 2011). This effect was greatly enhanced with paternal-inherited *arm-Gal4* without a responding transgene; interommadiatial bristles were also greatly reduced in number. A similar effect was seen for maternally-inherited *arm-Gal4* in the presence of paternal *UAS-lacZ*, but with no responding transgene, ommatidia and bristle number were moderately reduced. Expression of *GMR-Gal4* results in developmental defects and apoptosis in the *D. melanogaster* eye (Freeman, 1996; Kramer and Staveley, 2003). Although not as severe as the consequences of *GMR-Gal4* expression, the effects of *arm-Gal4* upon development of the eye observed in our study were significant.

Longevity assays comparing the consequences of *Gal4* expression in the presence of a benign responder, or with no responder, with the commonly-used *elav-Gal4* transgene and *arm-Gal4,* revealed a significant reduction in lifespan, from a median of 70 days to 42 and 48 days between the different drivers. The shorter lifespan of flies expressing *arm-Gal4* suggests that *D. melanogaster* development and regulation is severely impacted by the presence of *arm-Gal4*. Maternal and paternal expression of *arm-Gal4* did differ when no responder was present; median lifespans were 48 and 38 days, respectively.

Longevity assays of groups of *arm-Gal4*/*UAS-lacZ* (benign responder) critical class males revealed a much lower median life span for flies with maternally-inherited, compared to paternally-inherited *arm-Gal4*; the median lifespan was 40, compared to 60 days. The difference in lifespan indicates that parental inheritance of *arm-Gal4* affects expression patterns of *Gal4* by *arm* transcriptional control elements. The paternal *arm-Gal4,* in combination with a *UAS-lacZ* responder, demonstrated a partial rescue phenotype, suggesting that the negative effects of *arm-Gal4* are reduced when a responding transgene is present and *Gal4* is paternally inherited.

Generally, the *UAS-lacZ* responder is considered benign, as it is not native to *D. melanogaster*, and thus should not affect expression (Fischer et al., 1988; Brand and Perrimon, 1993; Phelps and Brand, 1998; Duffy, 2002). The results presented here suggest that *UAS-lacZ* may not be a benign responder and may influence development. Eye analysis and longevity assay results suggest that the presence of *arm-Gal4* in developing *D. melanogaster* can be detrimental, but the presence of a responding transgene may alleviate these effects.

Our experiments suggest that the *arm-Gal4* transgene and some so-called "benign" control transgenes may have significant effects. We suggest that, in all cases, the parental inheritance of *arm-Gal4* must be stated in experimental protocols, and that a description of "benign controls" is crucial. In a number of studies, the inheritance of *arm-Gal4* transgene has not been indicated

(Wang et al., 2005; Tsuda et al., 2007; Radyuk et al., 2009; Zid et al., 2009; Sen et al., 2010; Rahman at al., 2013); other studies have stated the parental inheritance of the flies (Rogina and Helfand, 2004). We advise caution in interpretation of experiments exploiting *arm-Gal4*, and other *Gal4* transgene(s), and emphasize the importance of controls and consideration of inheritance during experimental design.

Conflicts of interest

The authors declare no conflicts of interest.

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